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Aggregate dermal exposure to cyclic siloxanes in personal care products: Implications for risk assessment

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ABSTRACT

Consumers who use personal care products (PCPs) are internally exposed to some of the organic components present of which some may be detected in exhaled air when eliminated. The aim of this study was the quantitative determination of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) in end-exhaled air to study dermal absorption of substances in PCPs. We exposed the forearm of fifteen healthy volunteers for 60 min to pure D4 or D5 and to commercial products containing D4 and D5. Inhalation uptake was kept to a minimum by keeping the forearm in a flow cabinet during dermal exposure and supplying filtered air to the breathing zone of the volunteer during the post-exposure period. End-exhaled air was collected using a breath sampler (Bio-VOC), transferred to carbograph multi-bed adsorbent tubes and analyzed by thermal desorption gas chromatography mass spectrometry (TD-GC-MS). In the end-exhaled air of non-exposed volunteers background concentrations of D4 (0.8–3.5 ng/L) and D5 (0.8–4.0 ng/L) were observed. After exposing the volunteers, the level of D4 and D5 in end-exhaled air did not or barely exceed background concentrations. At t = 90 min, a sharp increase of the D4/D5 concentration in end-exhaled air was observed, which we attributed to the inhalation of the substances during a toilet visit without using inhalation protection devices. When this visit was taken out of the protocol, the sharp increase disappeared. Overall, the results of our study indicate that dermal absorption of D4 and D5 contributes only marginally to internal exposure following dermal applications. As in our study inhalation is the primary route of entry for these compounds, we conclude that its risk assessment should focus on this particular exposure route.

1. Introduction

Consumers use personal care products (PCPs) to clean, refresh and decorate their bodies. Some products are used on a daily basis, whereas others are used less frequently (Biesterbos et al., 2013; Wu et al., 2010).

When multiple products are used simultaneously, consumers may be exposed to the same substance through different sources and routes, also referred to as aggregate exposure (Lorenz et al., 2011; von Goetz et al., 2010). Cyclic siloxanes, such as octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), are added to PCPs as emollients or solvents (Johnson et al., 2011; Scientific Committee on Consumer Safety (SCCS), 2010) in many different products throughout the world (Dudzina et al., 2014; Horii and Kannan, 2008; Lu et al., 2011; Wang et al., 2009). D5 and to a lesser extent D6 were observed to be the predominant substances, whereas D4 was found in smaller amounts. The use of PCPs is the primary source of exposure to cyclic siloxanes (Health Canada, 2008). Therefore, the Scientific Committee on Consumer Safety in Europe and the Cosmetic Ingredient Review Expert Panel in the US assessed the health implications of the use of cyclic siloxanes processed in PCPs (Johnson et al., 2011; Scientific Committee on Consumer Safety (SCCS), 2010). Both committees concluded that cyclic siloxanes are safe with regard to the present practices of use and their concentrations in PCPs.

Abbreviations: D4, octamethylcyclotetrasiloxane; D5, decamethylcyclopentasiloxane; EI, electron impact; LOQ, limit of quantification; PBPK model, physiologically based pharmacokinetic model; PCPs, personal care products; TD-GD-MS, thermal desorption gas chromatography mass spectrometry.

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The substance specific properties of D4 and D5 (e.g. high vapor pressure) and the fact that PCPs are mainly applied to the skin, point towards inhalation and dermal uptake as the dominant determinants of the internal dose. The retention of inhaled labeled D4 was 5–6%, when rats were exposed to single and multiple dosages of ^{14}C-D4 (7, 70, 700 ppm) (Plotzke et al., 2000a). A physiologically based pharmacokinetic (PBPK) model was developed and successfully described the data presented above (Andersen et al., 2001). The PBPK model was extended with a pharmacodynamic model for hepatic enzyme induction by D4 (Sarangapani et al., 2002) and used to describe the tissue dosimetry, plasma concentration, and clearance in the rat following inhalation, dermal, oral, and intravenous exposure (Sarangapani et al., 2003). Furthermore, 12 healthy volunteers inhaled 122 μg/L D4, resulting in a mean intake of 137 ± 25 mg (Utell et al., 1998).

When male and female rats were exposed to single or repeated doses of 7 or 160 ppm ^{14}C-D5, lung retention was rather low (4–5% for single exposure and 8–10% for repeated exposures) (Tobin et al., 2008). Reddy and co-workers developed an inhalation PBPK model, using human and rat exposure data (Reddy et al., 2008). An additional compartment was added to describe the presence of D5 bound to blood proteins, indicating that not all D5 is freely available for biotransformation and elimination.

Zarebra and co-workers investigated the dermal absorption of neat D4 in human skin, using the human skin/nude mouse model (Zareba et al., 2002). Under semi-occluded conditions, only 1.1% of the applied dose was absorbed while a large fraction of D4 (95%) evaporated from the skin. A PBPK model was developed to determine the dermal uptake of D4 and D5 after application of these substances to the skin of axilla of human volunteers (Reddy et al., 2007). The percentages of the applied dose that reached the systemic circulation were calculated to be 0.12% and 0.30% for D4 in men and women, respectively, and 0.05% for D5 in both sexes. In a study on the *in vitro* and *in vivo* dermal absorption of ^{14}C-D4 and ^{14}C-D5, the majority of the applied substances volatilized before being absorbed (Jovanovic et al., 2008). Only small percentages of the applied dose were absorbed (0.5% D4 and 0.04% D5 *in vitro* and <1.0% D4 and 0.2% D5 *in vivo*). Despite these low absorption fractions, dermal exposure cannot be considered insignificant as dermal application of PCPs is the primary source of consumer exposure (Health Canada, 2008) and the applied products contain large quantities of D4/D5 (Dudzina et al., 2014; Horii and Kannan, 2008; Lu et al., 2011; Wang et al., 2009).

Assuming dermal absorption fractions of 0.5% for D4 and 0.04% for D5, Dudzina and co-workers calculated theoretical maximum internal doses after dermal application of PCPs that amount to 0.054 and 0.49 mg/capita/day, respectively, in a European population (Dudzina et al., 2014). Furthermore, the internal doses of D4 and D5 were determined in blood and exhaled air after dermal application of ^{13}C-labeled D4 and D5 to the axilla of human volunteers (Plotzke et al., 2000b, 2002). The average D4 concentrations ranged from 0.57 to 5.67 ng/g in blood and corresponded to values in exhaled air of 111 ng/L (women) and 30 ng/L (men). The D5 levels ranged from 0.61 to 1.41 ng/g in blood and from 347 to 2315 ng/L in exhaled air. Plotzke and colleagues (Plotzke et al., 2000b, 2002) investigated the internal dose after a single application of D4 or D5, but in reality consumers tend to use several products simultaneously, leading to aggregate exposure.

The concentration of a component in end-exhaled air reflects the blood concentration, which is considered to be the most reliable estimate of the internal dose for many substances (Angerer et al., 2007). However, sampling of blood is invasive and should be limited to a minimal number of samples. In contrast, the collection of end-exhaled air samples, using canisters, bags or glass tubes, is non-invasive. Because of easy accessibility repeated end-exhaled air samples can be obtained in a short period of time without causing much of a burden to the study participant (Alonso and Sanchez, 2013). D4 and D5 are excellent candidates for detection in end-exhaled air, because of their high vapor pressure and low blood:air partition coefficients. Therefore, we aimed to quantify the dermal uptake of D4 and D5 after dermal application of two PCPs (i.e. night cream and deodorant) and to subsequently investigate the internal consumer exposure to both D4 and D5 using end-exhaled air as a biological medium for sample collection.

2. Materials and methods

2.1. Chemicals and test substances

Octamethylcyclotetrasiloxane (98% D4; CAS 556-67-2) and decamethylcyclopentasiloxane (97% D5; CAS 541-02-6) were obtained from Sigma-Aldrich (St. Louis, MO, United States). A commercially available night cream (50 mL) and deodorant (40 mL) were purchased from an online retailer. According to the manufacturer, the night cream contained approximately 25% of D5 and 0.3% of D4. The deodorant contained approximately 30% D5 and 0.3% D4. ^{13}C-labeled D4 and ^{13}C-labeled D5, used as internal standards, were purchased from Dow Corning (Midland, MI, United States). Methanol (99.9%; CAS 67-56-1) supplied by Boom (Meppel, The Netherlands) was used to dissolve ^{13}C-labeled D4 or D5 and unlabeled D4 or D5 for the preparation of standards.

2.2. Study design

Fig. 1 provides an overview of the study design. We recruited 15 volunteers using information folders, bulletin boards and word of mouth. We included volunteers above 18 and not older than 70 years of age, with good general health. Volunteers were excluded from the study when they were using prescribed medication, were suffering from a skin disease (e.g. psoriasis or eczema) or worked occupationally with PCPs. Several animal studies showed that exposure to D4 may lead to a disruption of the reproductive cycle of a female rat (Johnson et al., 2011). Therefore, D4 was classified as a reprotoxic substance (Scientific Committee on Consumer Safety (SCCS), 2010). We decided to exclude female volunteers who were pregnant or not taking birth control measures.

The volunteers participated in a series of experiments, during which the forearm was exposed to D4 or D5 as a pure substance or as ingredient of a PCP (i.e. night cream and deodorant) for one hour. When the substance was removed, the volunteer provided several end-exhaled air samples over a time period of five hours to monitor the internal D4 or D5 concentration. A more detailed description of the experiments is provided below.

2.2.1. Baseline

Before the start of the study, all volunteers (N = 15) completed a questionnaire and a 24 h diary, which were used to assess their PCP usage pattern prior to the baseline experiment. Subsequently, the volunteers visited our laboratory. During this first visit, we collected duplicate samples of end-exhaled air to study the baseline (normal) excretion of D4 and D5. At this point in the study, the volunteers were not restricted regarding their PCP use.

2.2.2. Control experiments

To study the contribution of a background exposure to D4 and D5, we performed control experiments for some volunteers (N = 6). The experiment was executed as if it was an exposure experiment, but we did not administer D4 or D5 to the forearm of the volunteer. Instead, D4 or D5 was applied to an artificial arm (graduated cylinder wrapped with filter paper), placed next to the arm of the volunteer to determine the presence of D4 and D5 in end-exhaled air, without a dermal source. The participants were asked to refrain from the use of PCPs 24 h prior to the start of the experiments. However, they were allowed to brush their teeth using toothpaste provided by us. According to the ingredients list, this toothpaste was free of D4 and D5.

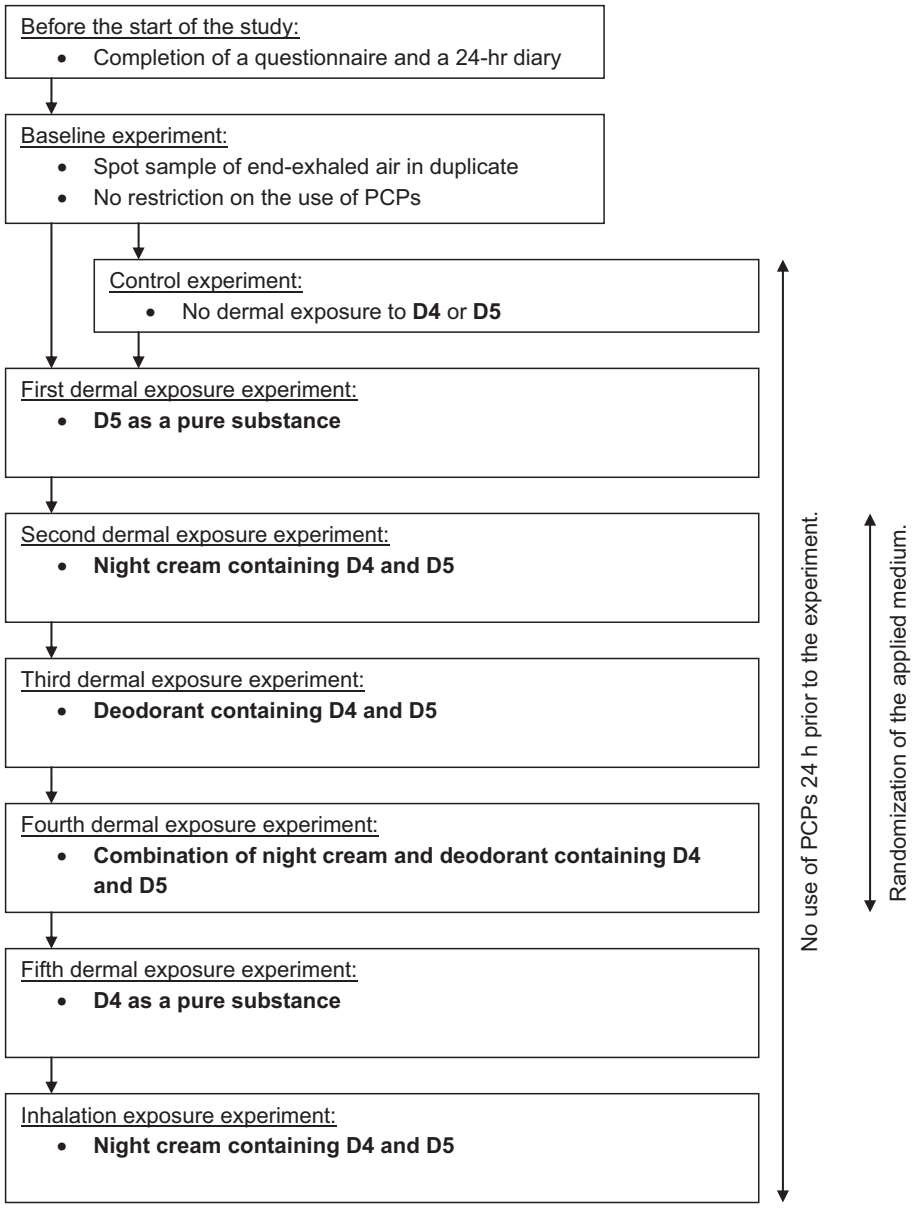

Fig. 1. Overview of the study design.

2.2.3. Dermal exposure experiments

We started a series of five exposure experiments in which each of the volunteers was dermally exposed to D4 and/or D5 in the following different media: pure substance, night cream, deodorant (stick) or a combination of the latter two. Aiming for an accumulated exposure of approximately 15 mg of D4 or D5 per cm^2, we applied an estimated dose of 2.5 mg of D4 or D5 per cm^2 to the forearm every 10 min over a total exposure period of 1 h to create a surplus of D4 and D5, and prevent back diffusion from the skin. We measured the surface area of the forearm of the volunteer to determine the amount of substance needed to achieve this total dose. The net applied dose was determined by weighing the applied substance before the start of the experiment and at the end, by recovering the residue from the arm. The content of D4 in night cream and deodorant was approximately two orders of magnitude lower than the content of D5, implying that the total accumulated dose of D4 after application of these products was approximately 0.15 mg per cm^2. The resulting applied amount in the experiments with formulated D4 was too low to be detected after dermal absorption and therefore not further studied. This protocol does not reflect typical use of a night cream or deodorant. We calculated the minimum level of D4 and D5 in end-exhaled air needed for detection with our analytical method. Subsequently, we calculated the corresponding systemic dose of D4 and D5 and derived the required amount and surface area of the products required to achieve this systemic dose.

To prevent inhalation exposure, the participant was sitting with his or her forearm inside a flow cabinet in our laboratory (Fig. 2A). To monitor the ambient air concentration and potential inhalation during the exposure and post-exposure period, the participant carried a head-set (Fenix Environmental, Umeå, Sweden) with two sampling heads that were placed in the breathing zone slightly above the nose (Lindahl et al., 2009). The sampling heads were equipped with mini ATD tubes loaded with Tenax TA (Fenix Environmental, Umeå, Sweden) that provided time-weighted average concentrations of D4 and D5 in ambient air during the exposure and post-exposure periods.

To obtain a clear kinetic profile exposure was ceased after 1 h by removing the residual substance using a spatula and washing the arm with water and soap (without D4/D5 on the ingredient list) inside the flow cabinet. To prevent inhalation of D4 and D5 from ambient air after cessation of the exposure period, a fume hood was placed over the head of the participant (Fig. 2B), which supplied a constant

Fig. 2. A: A participant with his lower arm inside the flow cabinet during administration. B: A participant after cessation of exposure with the fume hood placed over his head.

downstream flow of filtered air. The fume hood was custom made, using a 3M Jupiter air stream device connected to a 3M Versaflo Headtop and equipped with two 3M A2BEKP R filters (Biesheuvel Techniek, Wijchen, The Netherlands). Throughout the post-exposure period (5 h), the volunteer was seated behind a desk in an office space. Every hour the participant was asked to visit the toilet to provide a urine sample, during which no attempt was made to prevent inhalation of ambient air. At some point during the exposure experiments to D5 as a pure substance, preliminary results showed a sharp increase in the end-exhaled air concentration directly after the first toilet visit, i.e. at the end of the 1 h exposure period. After this discovery, this toilet visit was removed from the protocol.

2.2.4. Inhalation experiments

Finally, we conducted exposure experiments without the prevention of inhalation, as the results of the previous experiments showed that inhalation instead of dermal uptake appeared to be the major route of exposure. The volunteer was seated inside a toilet room of approximately 9 m^3. Four grams of night cream was applied to the forearm of the volunteer. After 5 min, the night cream was removed and the forearm washed with water and soap. The volunteer remained seated for another 10 min, thereafter inhalation exposure was terminated by moving the volunteer from the exposure room to the office where the participant was seated underneath the fume hood.

2.3. Sample collection

Before, during and after exposure, end-exhaled air was collected at predetermined intervals (Fig. 3). The participants were asked to exhale completely into a disposable cardboard mouthpiece that was fitted to a 141.5 mL Bio-VOC container (Markes International, Llantrisant, United Kingdom). In total, 17 end-exhaled air samples were collected in duplicate, resulting in a total of 34 samples per person per experiment. The collection times of the samples differed between those exposure experiments using pure substance and those using a night cream or deodorant. We distributed the sampling times more evenly when applying a night cream or deodorant, as there might be delayed absorption due to a matrix effect. Immediately after collection of the sample, the organic compounds were transferred to a 1/4″ × 3.5″ Stainless Steel tube filled with Carbograph 2TD 60/80 and Carbograph 1 TD 60/80 (CAMSCO, Houston TX, United States).

We collected urine samples before the start and after the end of the exposure experiments to monitor the elimination of both D4 and D5 and their metabolites after dermal absorption of the applied products (Fig. 3). In the post-exposure period, we asked the participants to visit the toilet every hour, leading to a minimum of 6 samples per volunteer per experiment. During the toilet visits, the volunteer was not placed underneath the fume hood. The urine samples were stored in Vacuettes (Greiner Bio-One, Alphen a/d Rijn, The Netherlands) at −20 °C. These results are not reported in the present paper.

2.4. Sample analysis

We prepared an internal standard solution of 5 ng/μL ^{13}C-labeled D4/D5 in methanol, to quantify end-exhaled air samples. Prior to analysis, 2.5 ng of ^{13}C-labeled D4 /D5 (0.5 μL) was loaded on the ATD tubes using a loading rig (Markes International, Llantrisant, United Kingdom). The ATD tube was connected to the loading rig, the internal standard solution was injected via a syringe and the tube flushed with helium 5.0 (Linde Gas, Schiedam, The Netherlands) at a flow of 50 mL/min for 3 min to remove the methanol.

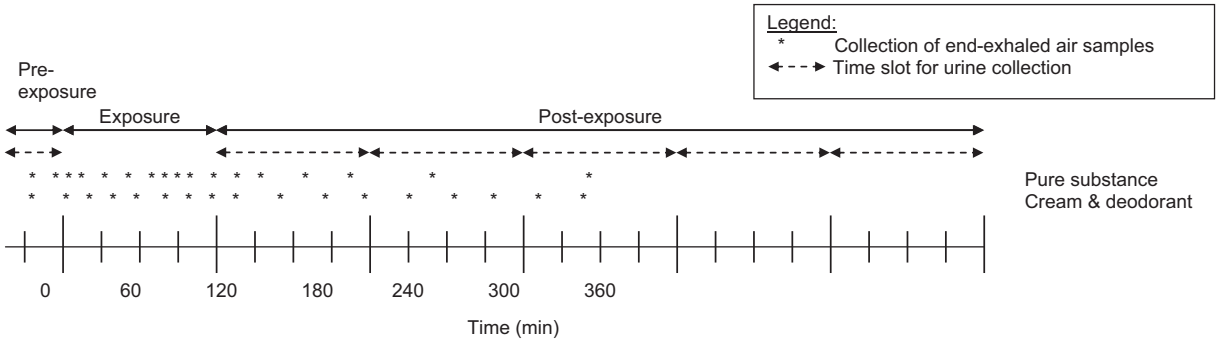

Fig. 3. Overview of the time points used for the collection of end-exhaled air and urine samples.

The samples were analyzed using thermal desorption gas chromatography mass spectrometry (TD-GC-MS). The analytical instrument consisted of a thermal desorption unit and an auto sampler (Unity 2 and Ultra 2, Markes) coupled to a gas chromatograph mass spectrometer (Focus and ISQ, Thermo) using electron impact ionization (EI). The ATD tubes were positioned in the autosampler and subsequently desorbed at 275 °C for 15 min. The analytical column was a 30 m Rxi-5 MS (0.25 mm i.d., 0.5 μm film thickness, Restek). The carrier gas was helium 5.0. The GC oven temperature was 50 °C; hold 5 min; 10 °C/min to 150 °C; and 30 °C/min to 250 °C, hold 2 min. The transfer line was kept at 250 °C and the ion source at 250 °C. The ions monitored were *m/z* 281 for D4, 355 for D5, 285 for ^{13}C-labeled D4 and 360 for ^{13}C-labeled D5, respectively. The dwell time was 0.4 s.

The limit of quantification (LOQ) in end-exhaled air was 2.1 ng/L for D4 and 1.4 ng/L for D5. The calibration curve included seven standard solutions with a concentration range of 0–10 ng/μL. A more detailed description of the analytical method can be found elsewhere (Biesterbos et al., 2014). The urine samples were stored for future analysis.

2.5. Study approval

The study was approved by the Regional Committee on Research involving Human Subjects Arnhem-Nijmegen (registration number: 2011/131). Additional information on procedures used to ensure the safety of the volunteers can be found in Supplementary information I.

3. Results

3.1. Baseline

We included 15 participants with a mean age of 42 ± 18 years, including ten women. Table 1 provides an overview of the median concentrations of D4 and D5 measured in end-exhaled air of our volunteers during the baseline measurements. The raw data are presented in Supplementary information II. The individual use patterns of PCPs during the 24 h prior to the baseline measurement were reported in a 24 h diary and are presented in Supplementary information III.

3.2. Control experiments

Six volunteers participated in the control experiments. The mean D4 and D5 concentrations in end-exhaled air and the mean time-weighted average D4 and D5 concentration in ambient air measured during the control experiments are presented in Fig. 4. The detailed results for each volunteer are presented in Supplementary information IV–V. The measured concentrations in end-exhaled air ranged from 0.8 to 3.5 ng/L (D4) and from 0.8 to 4.0 ng/L (D5). We considered this to be the background level. The mean time-weighted average ambient air concentrations of D4 were 4.8 μg/m^3 during exposure and 1.3 μg/m^3 during the post-exposure period. For D5 the concentrations were 1.3 μg/m^3 during exposure and 4.3 μg/m^3 post-exposure.

3.3. Dermal exposure experiments

Table 2a–c provides an overview of the mean net amount of substance or product dermally applied and the resulting mean net dermally applied dose of D4 and D5. The raw data of each volunteer are presented in Supplementary information VI. The net applied dose did not reach 15 mg/cm^2, indicating that a residue was removed at the end of the exposure period (1 h).

Table 1
Median baseline concentrations of D4 and D5 in end-exhaled air of all participants after normal use of PCPs.

	Median (min–max)	
Participants	D4 (ng/L)	D5 (ng/L)
Men (N = 5)	4.3 (2.7–11.8)	2.3 (1.6–27.1)
Women (N = 10)	7.0 (1.9–44.8)	5.7 (2.1–44.4)
All (N = 15)	6.6 (1.9–44.8)	4.4 (1.6–44.4)

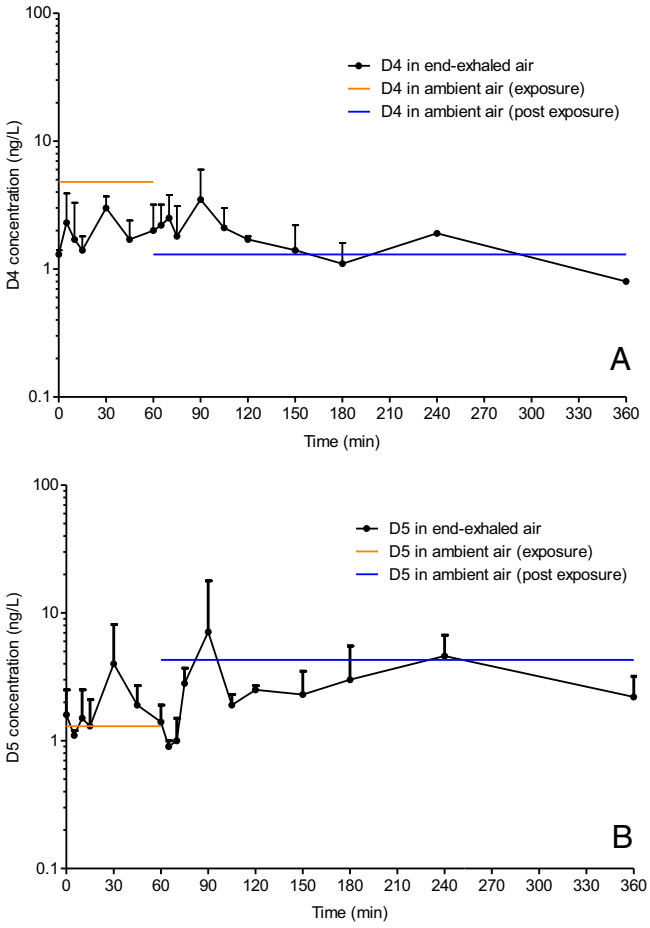

Fig. 4. Mean (±SD) D4 (A) and D5 (B) concentrations in end-exhaled air (ng/L) and mean time-weighted average D4 and D5 concentrations in ambient air (μg/m^3) during control experiments (N = 3 for each substance).

In total, 29 exposure experiments were performed, during which the volunteers were exposed to:

A. D5 as a pure substance (N = 13).
B. D5 as a pure substance, without the toilet visit at t = 90 min (N = 3).
C. Night cream containing D4 and D5 (N = 4).
D. Deodorant containing D4 and D5 (N = 1).
E. Combination of night cream and deodorant (N = 2).
F. D4 as a pure substance, without the toilet visit at t = 90 min (N = 6).

Fig. 5 provides an overview of registered D4 and D5 patterns observed in end-exhaled air following exposure to different media (A–F). Results for each individual are presented in Supplementary information VII–XII. In general, the D4 and D5 concentrations in end-exhaled air showed a fluctuating pattern, which included increases and decreases in the concentrations during both the exposure and post-exposure periods. The concentrations measured did not or only barely exceed the maximum background concentrations, which were determined during the control experiments. The peak levels of D4 in end-exhaled air of different individuals occurred during exposure and ranged from 7.5 to 280 ng/L. The peak levels of D5 in end-exhaled air occurring during both the exposure and the post-exposure periods ranged from 3.8 to 605 ng/L.

Table 2
Overview of net amount of D5 applied using pure substance, a night cream and a deodorant (a), a combination of night cream and deodorant (b) and the resulting net applied dose D5. Overview of applied net amount of D4 pure substance (c) and the resulting net applied dose D4. Data are presented as mean (SD).

a.

Applied D5	Exposed surface forearm (cm^2)	Amount (g)	Dose (mg/cm^2)
D5 pure substance (N = 13)	805 (112)	4.8 (1.2)	5.9 (1.7)
D5 pure substance (no toilet visit at t = 90 min) (N = 3)	822 (209)	6.1 (1.3)	7.5 (1.2)
Night cream (N = 4)	818 (83)	14.4 (5.5)	4.4 (1.6)
Deodorant (N = 1)	689	5.3	2.3

b.

Applied D5	Exposed surface forearm (cm^2)		Amount (g)		Dose (mg/cm^2)		
	Deodorant	Night cream	Deodorant	Night cream	Deodorant	Night cream	Total
Night cream and deodorant (N = 2)	795 (34)	781 (28)	16.6 (4.0)	9.0 (0.8)	5.3 (1.1)	3.4 (0.1)	8.7 (1.2)

c.

Applied D4	Exposed surface forearm (cm^2)	Amount (g)	Dose (mg/cm^2)
D4 pure substance (N = 6)	832 (147)	8.6 (1.4)	10.1 (1.9)

3.4. Inhalation experiments

Fig. 6 presents the mean D5 concentrations in end-exhaled air and the mean time-weighted average ambient air concentrations measured during the inhalation experiments (N = 3). The results for each volunteer separately are presented in Supplementary information XIII. The highest concentration of D5 in end-exhaled air was measured during the stay in the toilet room, between 5 and 10 min after the application of night cream. This concentration ranged from 1000 to 1500 ng/L. As no attempt was made to remove volatilized substance form the breathing zone, the time-weighted average ambient air concentration was approximately two to three times higher compared to the average ambient air concentration during the dermal exposure experiments (Section 3.3). When the same experiment was performed inside the toilet room but without the application of night cream, the D5 concentration in end-exhaled air was approximately 4 ng/L, which is similar to that in the control experiments. The D5 concentration in ambient air was approximately 16 $\mu g/m^3$ (data not shown).

4. Discussion

Using the experimental setup described in this paper, we could not confirm significant dermal uptake of D4 and D5 after topical application of the neat substance or PCPs on the skin of healthy volunteers. We were also able to investigate internal consumer exposure to both D4 and D5 present in PCPs after dermal exposure.

4.1. Baseline

The results of the baseline measurements showed that some volunteers (i.e. volunteer 2, 4, 5, 11, 13 and 15) had a ratio of D4/D5 that exceeded unity. This is remarkable, since nowadays D5 is the primary siloxane ingredient used in personal care products and D4 is phased out in several products. Similar findings were reported by Hanssen and co-workers (Hanssen et al., 2013) who analyzed blood samples from 94 postmenopausal women and 17 pregnant women and found that D4 was the dominant compound in plasma of both cohorts. The authors suggested that the lower dermal uptake and higher lipophilicity of D5 relative to D4 (log Kow = 8.03 versus 6.49, respectively) (Xu and Kropscott, 2012) could explain the higher observed D4 plasma concentrations.

4.2. Dermal exposure experiments

We included 15 healthy volunteers to participate in the dermal exposure experiments. Our intention was to expose all volunteers according to the protocol presented in Fig. 1. However, during the study preliminary results indicated that the dermal absorption of the applied D4 and D5 was very low. For ethical reasons, we decided to discontinue the study. As a result, not all volunteers were exposed to every product leading to low numbers of exposed volunteers in some groups (e.g. N = 1 for deodorant).

Dermal exposure ended after 60 min by removal of the applied substance from the skin surface using water and soap. Under normal circumstances a consumer will not remove a night cream or deodorant after one hour, extending the time that D4 and D5 is present on the skin and available for absorption. In theory, this means that we could have underestimated the total amount of D4 and D5 absorbed through the skin. However, presumably the amounts applied by consumers are too low to be absorbed in quantities that could be detected with our analytical method. Due to the high vapor pressure of both substances the longer availability on the skin of both substances is not relevant, as both substances evaporate quickly.

Shortly after the end of exposure, the volunteer provided three end-exhaled air samples (t = 65, t = 70, and t = 75 min). During these actions the forearm was kept inside the flow cabinet. The surface air velocity of 0.35–0.75 m/s had little impact on the emission of substances present in diaper cream. However, it did influence the emission of substances from a liquid fragrance (Wang et al., 2011). As the air velocity inside the flow cabinet was relatively low (0.41 ± 0.03 m/s) and the applied amounts of neat D4 and D5 were large, we consider the losses to be negligible.

Subsequently, the fume hood was placed over the head of the volunteer. Before the collection of the next end-exhaled air sample (t = 90 min), the volunteer visited the toilet for the collection of a urine sample and was invited to remain seated underneath a fume hood behind a desk in an office space for the rest of the observation period. It was not feasible to use the fume hood during the toilet visit. Several graphs showed a sharp increase of the concentration of D5 in end-exhaled air at this time point (t = 90 min). The mean D5 concentration in end-exhaled air at t = 90 min was 69.6 ng/L with the toilet visit and 5.7 ng/L without the toilet visit (compare Fig. 5A and B). The sharp increase in D5 concentration at t = 90 min is most likely explained by the inhalation of the substance during the toilet visit. The source is most likely evaporation of a residue from the treated skin surface (i.e. not completely removed or back-diffused from the skin to the air). At

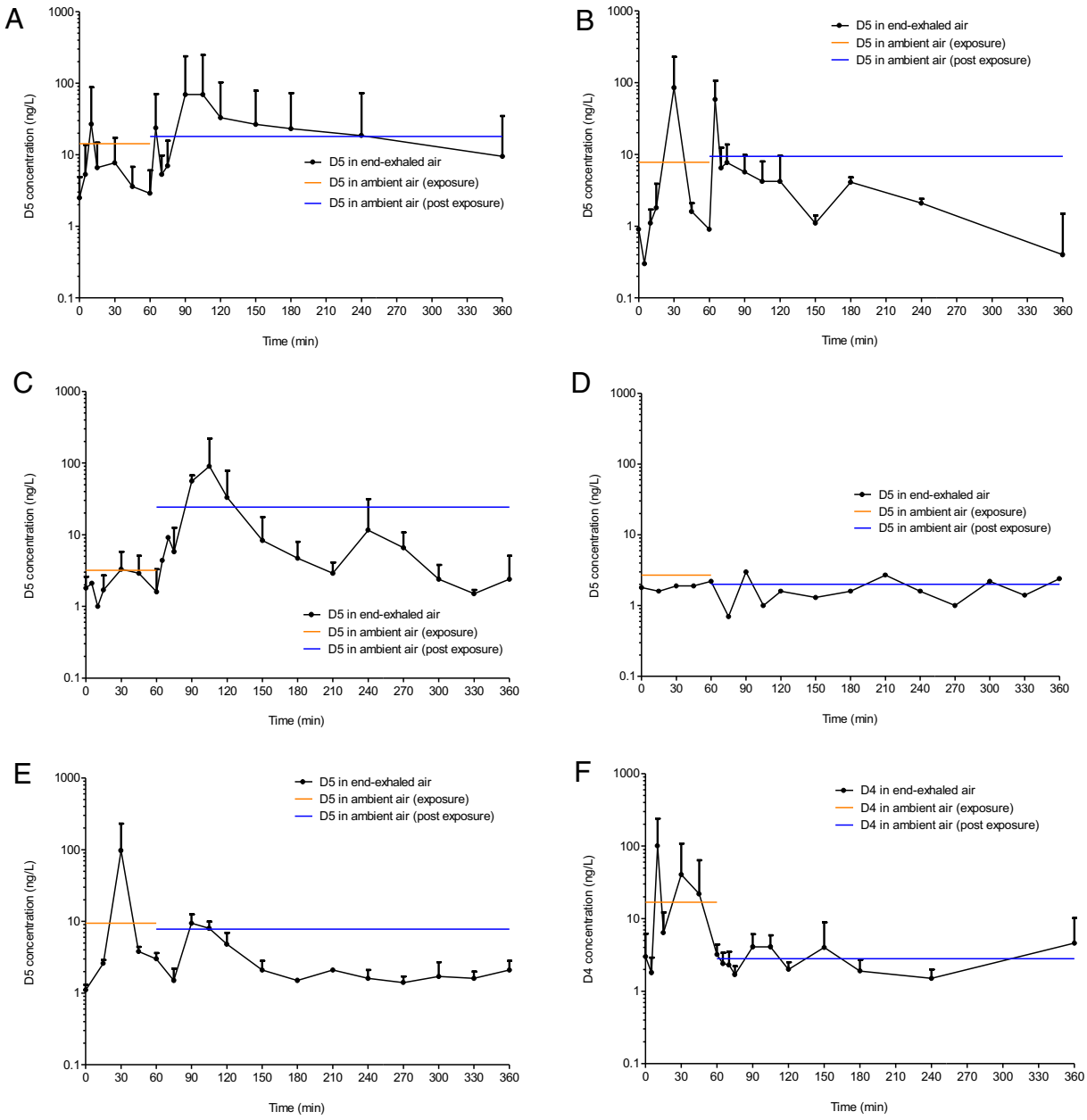

Fig. 5. Mean D4 or D5 concentrations in end-exhaled air (ng/L) and mean time-weighted average D4 or D5 concentrations in ambient air (μg/m^3) after dermal exposure to D5 as a pure substance (N = 13) (A), to D5 as a pure substance without the toilet visit at t = 90 min (N = 3) (B), a night cream (N = 4) (C), a deodorant (N = 1) (D), a combination of night cream and deodorant (N = 2) (E) and D4 as a pure substance without the toilet visit at t = 90 min (N = 6) (F).

other time points during the experiments, we occasionally observed similar unexpected and sudden increases of D4 and D5 concentrations and suggest that inhalation exposure is the most probable explanation in those cases. As the pattern showed similarities with the peak at t = 90 min, we assume that unintended vapor releases may have contributed to these peaks, despite the fact that the volunteers were placed with their forearm inside a flow cabinet (exposure) and underneath a fume hood (post-exposure).

The maximum concentration of D4 (7.5–280 ng/L) in end-exhaled air of different individuals occurred during the exposure period. The maximum concentration of D5 (3.8–605 ng/L) occurred during the exposure and post-exposure periods. These concentrations are well within the range of results earlier reported by Plotzke and colleagues (Plotzke et al., 2000b, 2002). They observed maximum concentrations of D4 in exhaled air ranging from 30 to 111 ng/L, after the application of 1.0 or 1.4 g of ^{13}C-labeled D4 to the axillae, at 60 min after exposure. When applying similar quantities of ^{13}C-labeled D5 to the axillae, the authors observed peak levels of D5 in end-exhaled air between 15 and 60 min after exposure, ranging from 347 to 2,315 ng/L. Plotzke and coworkers did not report measures to prevent inhalation during dermal application of ^{13}C-labeled D4 (Plotzke et al., 2000b). During the application of ^{13}C-labeled D5 volunteers were instructed to breathe from a clean air source (Plotzke et al., 2002). The exact time point and duration of usage of the clean air source were not specified, nor did they determine concentrations of ^{13}C-labeled D5 in ambient air. After the application of the substance a large fraction evaporates and as no information is provided on the time point and method of cessation of exposure, evaporation may have continued. Due to this supposedly limited prevention of inhalation exposure and the fact that the peak concentrations D4 and D5 in end-exhaled air in both studies are similar to our findings, we suspect that the results reported by Plotzke and colleagues do not exclusively represent dermal absorption but most probably reflect total

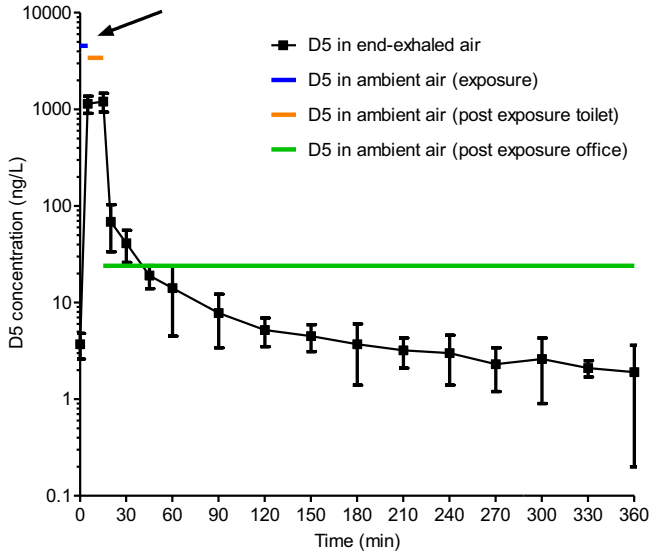

Fig. 6. Mean (±SD) D5 concentrations in end-exhaled air (ng/L) and mean time-weighted average D5 concentration in ambient air (μg/m^3) during inhalation experiments (N = 3). The arrow indicates the D5 concentration in ambient air during exposure.

exposure from both inhalation and dermal absorption pathways. If this is true, the dermal PBK model developed by Reddy and colleagues (Reddy et al., 2007) was parameterized incorrectly because D4 and D5 concentrations in exhaled air by Plotzke and co-workers were attributed to the dermal uptake route only, whereas inhalation probably also played a significant role.

4.3. Ambient air concentrations

We provided the volunteers with a headset to assess potential inhalation exposure to D4 and D5 during the control and exposure experiments. A time-weighted average D4 or D5 concentration was measured in a laboratory environment during the exposure period. Next, the volunteer was instructed to remain seated underneath a fume hood behind a desk in an office space, thus the time-weighted average concentration post-exposure reflects filtered air concentrations. The results fluctuated between the individual dermal exposure experiments and between the exposure and post-exposure periods within these experiments, but our results are within the range reported by previous studies that assessed normal background indoor concentrations of cyclic siloxanes (Hodgson et al., 2003; Kaj et al., 2005; Pieri et al., 2013; Shields, 1996; Wu et al., 2011).

4.4. Dermal uptake versus inhalation exposure

Our results indicate that the dermal uptake route of D5 is much less important than previously assumed. We even hypothesize that the dermal uptake route may be insignificant compared to the inhalation route. To explore this issue further, we applied the PBK model of Reddy and coworkers (Reddy et al., 2007) to estimate the concentration of D5 in exhaled air after inhalation exposure only, ignoring the possible contribution of dermal uptake. In inhalation experiments (Section 3.4), we measured the ambient D5 concentrations present in the toilet area before and during dermal exposure to a night cream. The mean D5 concentrations in ambient air were approximately 14 μg/m^3 (pre-exposure) and 4500 μg/m^3 (exposure). These concentrations served as input for the human PBK model, reflecting a minimum and maximum exposure scenario for the toilet visit. The modeled exhaled air concentrations were approximately 10 ng/L and 3200 ng/L, respectively, for these scenarios. The D5 concentrations measured in end-exhaled air at t = 90 min varied between 10 and 600 ng/L and are thus within the range of these modeled exhaled air concentrations. These results indicate that the measured concentrations in exhaled air can be explained based on inhalation exposure only, confirming our hypothesis that dermal uptake plays a minor role. We conclude that risk assessment of D5 should focus on inhalation exposure since the contribution of dermal absorption of D5 to internal exposure is only marginal.

5. Conclusions

Overall, the results of our dermal exposure experiments using pure D5, pure D4, a night cream, a deodorant and a combination of the latter two indicate that concentrations of D4 and D5 eliminated in end-exhaled air cannot be discriminated from background levels observed following the non-use of PCPs for 24 h. When applying PCPs, such as a night cream or deodorant containing cyclic siloxanes (D4/D5), inhalation and not dermal exposure is the primary pathway of uptake. Therefore, it is important to take inhalation exposure into account when performing aggregate exposure assessments to dermally applied D4, D5 or substances with physicochemical properties similar to these volatile cyclic siloxanes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.10.017.

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